

Molecular Analysis of Heritable Mouse Mutations

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Germ-line mutations of the mouse have for years comprised one class of biological markers for mammalian reproductive and developmental toxicology. Understanding the molecular nature of mutations and the mechanisms by which mutations are translated into specific (and often complex) phenotypes, however, still looms as a major goal of mammalian biology. Molecular genetic analysis of heritable mouse mutations constitutes a significant, experimentally malleable strategy for relating genomic DNA structure to genic expression and function in mammals. The integrated use of recombinant DNA technology, which allows both the identification and analysis of expression of single genes, and classical genetic and cytogenetic analysis, which allow the important correlation between basic DNA defects and the organismic consequences of such defects, has been crucial to this strategy. Some of the approaches (e.g., specific-gene cloning, random-clone analysis of genomic regions, insertional mutagenesis) for studying the nature and effect of both mutations and their wild-type counterparts that have resulted from this integration of genetic analysis and molecular biology have been applied to many loci within the murine genome. Studies of the nature and effects of a complex set of radiation-induced mutations at the dilute-short ear (*d-se*) region of chromosome 9, a specific example of this type of integrated analysis, are discussed.

Introduction

The complexity of the mammalian genome is realized both in terms of its organization and structure at the DNA level and in terms of the myriad functions and diverse developmental processes that the genome specifies and regulates throughout the life cycle of the organism. For over 80 years, geneticists have identified and characterized hundreds of mutations of the laboratory mouse. In the context of biological markers for reproductive and developmental toxicology, these heritable mutations take on fundamental roles. Mutations have provided some basic insights into the composition and organization of the mammalian genome as well as into the roles specific genes play in normal development of the mouse. Mutations have to a large extent defined individual routes and pathways within the spatial/temporal continuum of normal development by creating subsets of abnormal phenotypes that can be easily discerned against the background of the wild-type genome.

The techniques of molecular biology clearly have added a new dimension to the study of mammalian genetics and heritable mouse mutations. Molecular tools, in combination with available genetic resources and methods of transmission and somatic-cell genetics, offer powerful approaches to elucidating the relation between DNA structure, DNA expression, and phenotype, as well as to understanding the genetic control of normal

developmental processes in mammals. Table 1 lists some research areas in the field of mammalian genetics that should benefit from combined molecular and genetic studies. It is beyond the scope of this paper to review each topic; instead, several of these avenues of inquiry and some of the roles that genetic and molecular analysis of heritable mutations of the mouse play in mammalian genetics are discussed.

Heritable Mouse Mutations in Mammalian Biology

Each spontaneous or agent-induced germ-line mutation of the mouse serves as a marker for a segment of DNA within the genome that has a specific function. Genetic analysis of a new variant may result either in establishment of allelism with a gene at a recognized locus or in placement of a new locus on the genomic map. The phenotype that is expressed serves as a marker for a particular, often adverse, effect on the normal anatomy, physiology, and fitness of the animal; these effects result from the altered structure and function of the corresponding segment of DNA. Consequently, the current linkage map of the mouse (*1*), which is comprised of hundreds of loci distributed among 20 chromosome pairs, represents a collection of markers defining subsets of genes that play specific developmental roles at various phases throughout the life cycle of the animal. These markers continue to expand in number; indeed, loci included in the current linkage map

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Table 1. Areas of mammalian genetics that should benefit from combined genetic and molecular analysis.

Basic organization and evolution of the mammalian genome
Spontaneous and induced germ-line mutations; genomic structure
Spontaneous and induced germ-line mutations; developmental effects
Identification and characterization of new genes; expansion of the genetic map
Genome instability; insertion-induced mutations
Nature and effect of dominant mutations
Nature and effect of pleiotropic mutations
Basis for "irregular inheritance" (variations in penetrance and/or expressivity)
Nature and effect of spontaneous and induced somatic mutations
Mechanisms of oncogenesis
Developmental regulation of DNA expression; temporal and tissue specificity
Environmental influences on genetic systems
Study of multigene families; origin, evolution, generation of diversity

probably represent only a small fraction of genes that will eventually be found within the mammalian genome (see "Molecular Analysis of Mutations at the Dilute-Short Ear (*d-se*) Region of Chromosome 9").

Heritable mutations have also been utilized as indicators, or end points, for testing the mutagenic effects of various physical and chemical agents on the mammalian germ line. A description of this use of mouse mutations as biomarkers in genetic toxicology is likewise beyond the scope of this paper (2). However, it should be emphasized that mutagenesis experiments are an important source of discovering mutations of diverse natures (depending on the mutagenic agent employed) that either define new genetic loci or provide alleles varying in degrees of pleiotropy and severity of effect at already existing, defined loci. Consequently, the use of heritable mutations as end points in agent testing is contributing significantly to a biological resource that can be exploited genetically, phenotypically, and molecularly to learn more about the structure and function of the mammalian genome, in addition to providing important information about what is harmful to the germ line and future generations.

Molecular analysis of heritable mouse mutations is a necessary step in relating mutational alterations in DNA structure with the resultant phenotypic manifestations. Such analysis should lead also to an understanding of the normal gene (and gene product) and to an understanding of the normal developmental process in which that gene is involved. Thus, genetic and molecular analyses, both of a mutation responsible for a detectable change in phenotype and of its corresponding wild-type DNA, can lead to an understanding of the nature of the mutation at the DNA level and, perhaps, to the characterization of a component of a specific process or pathway. This information, in turn, may help elucidate the molecular mechanisms of the primary process, namely how the genotype at a particular locus is translated into a specific, characteristic phenotype.

Molecular Approaches to Genetic Analysis in the Mouse

Specific-Gene Cloning

Isolation of cloned DNA sequences to provide molecular access to a genetic locus is an important prerequisite for initiating the molecular analysis of the nature and effects of mutated genes and their wild-type counterparts. Several approaches to this basic problem are now in use by numerous laboratories. One of these approaches, and perhaps the most direct, is the molecular cloning of a gene whose gene product is easily accessible and is at least partially characterized. For example, cDNA clones for abundant RNAs, such as globin mRNA in erythropoietic tissues, may be obtained with little purification of the RNA populations (3). Furthermore, if the protein product itself has been purified, specific antibody to that protein can be used in a variety of protocols, such as immunoprecipitation of polyribosomes, hybrid selection and immunoprecipitation of *in vitro* translation products, and screening of cDNA expression libraries, to identify cDNA clones associated with specific gene products (4-7). Likewise, if the amino acid sequence of at least part of the gene product of interest is known, unambiguous oligonucleotide probes may be designed for use as probes to select corresponding clones from cDNA libraries. Once a cDNA clone becomes available for these types of expressed genes, analysis of structural and regulatory features of the corresponding genomic DNA sequences can commence. For example, isolation of cDNA clones for the metabolic enzyme β -glucuronidase (GUS) by Palmer et al. (7) is particularly cogent in this context of genomic analysis of mouse mutations. Several structural and regulatory variants of the *Gus* complex on chromosome 5 have been identified by genetic analysis of inbred and wild mice over the years (8). Analysis of the genomic DNA within and around the *Gus* gene should lead to an understanding of the molecular basis for these variant phenotypes.

Random-Clone Analysis

Perusal of both the current linkage map of the mouse and the elegant descriptions of variant loci compiled by Green (9) makes it clear that many genetic loci are defined only by mutant phenotypes and have no easily discernible protein product (e.g., *d*, dilute; *se*, short-ear; *Sl*, Steel; *Hm*, hammertoe; *Ie*, eye-ear reduction; etc.). Obtaining molecular access to the wild-type and mutant forms of these types of loci presents a more difficult problem. In fact, it presents two problems: selection of DNA clones from the total genomic pool that map to the locus in question, and confirmation that such clones actually represent DNA sequences from the gene of interest. Generation and mapping of random DNA clones within the genome and the technique of insertional mutagenesis are two approaches that have evolved in response to these types of molecular access problems.

I have defined "random" DNA clones simply as segments of DNA not associated *a priori* with a specific gene product. These DNAs may be derived from coding or noncoding sequences, some may be unique sequence and some may be repetitive within the genome. For example, a bacteriophage library of mouse genomic DNA derived from the total genome, from flow-sorted chromosome preparations, or from microdissected segments of metaphase chromosomes all represent populations of random genomic clones. Map positions for unique-sequence clones can typically be obtained by analyzing linkage between known genetic loci and restriction-fragment-length-polymorphisms (RFLPs) identified by the DNA clone in question. Thus, mapping of random clones by standard procedures can place loci defined by DNA polymorphisms onto the genetic map, often close to otherwise inaccessible loci defined only by a mutant developmental phenotype. One can anticipate that as the placement of DNA markers onto the linkage map continues, more and more inaccessible loci will emerge near enough to a molecularly cloned sequence to allow the clone to be used for accessing the locus. [It should be noted, however, that an anonymous, random DNA clone mapping as close as 1 centimorgan (cM, 1% recombination) from a particular locus may be 1000 to 2000 kilobases (kb) of DNA away from the actual gene; this figure is based on current gross estimates of the relationship between recombination frequency and physical distance. Consequently, with current techniques, even this clone would not be a useful access point, since the physical distance from the clone to the locus of interest is very large.]

A useful example of this type of analysis is the mapping of endogenous retroviral sequences within the mouse genome (10–13). Integration sites of these retroviral sequences can be identified and mapped in relation to other loci by Southern blot analysis using virus-specific recombinant-DNA probes. The genomic, non-viral sequences flanking these endogenous viruses thus represent a type of random clone, permitting access to the area of the genome defined by the integration of that particular virus. The close association of the lethal yellow (A^y) mutation on chromosome 2 with the site of integration of an ecotropic retrovirus (14) provides a good example of how this type of random-clone analysis might be used to access the genomic DNA close to an otherwise inaccessible mouse mutation.

Random-clone analysis has been quite useful in initiating molecular analyses of entire chromosomal regions in which are found a number of mutations with specific phenotypic effects. Microdissection and microcloning of the proximal segments of chromosome 17 (15), for example, have resulted in the availability of a number of random clones that map to various areas within the approximately 15 cM mouse *t* complex (16). Genetic analysis over the years has associated phenotypes such as embryonic lethality, abnormal male transmission ratios, male sterility, recombination suppression, and tail abnormalities with this region of chromosome 17. Se-

lection of random microclones for this region and their subsequent mapping to subregions of the complex have been important initial steps toward the molecular characterization of the genes involved in the *t*-complex phenotypes that have both perplexed and fascinated mammalian geneticists for years (16).

Random-clone analysis of regions of the mouse genome associated with sets of radiation-induced deletion mutations is likewise a powerful tool for initiating the molecular analysis of entire chromosomal regions (and mutant phenotypes associated with those regions), as well as for identifying new genetic loci that play roles in normal development. Complementation analysis of sets of independently derived deletion mutations of varying length overlapping within a given region can generate functional maps for segments of chromosome around the primary selected mutation (17–19). In addition, large multilocus deletion mutations can be used to identify, from the total genomic pool, random cloned DNAs that map to a specific region of interest, completely bypassing more laborious standard transmission linkage (recombination) experiments employing cloned DNAs and either visible or biochemical marker loci. Once clones are obtained for a large region (defined, for example, by the longest deletion available for that region), sets of overlapping deletions can be used to map clones with respect to deletion breakpoints. This exercise results not only in an ordering of clones with respect to one another within a small segment of chromosome, but also in the discovery of correlations between specific cloned DNA sequences and observed biologic effects defined by genetic analysis of the mutations employed for mapping. This type of analysis, using the dilute-short ear (*d-se*) panel of radiation mutations as an example, is discussed in more detail below.

Insertional Mutagenesis

Another powerful approach for providing molecular access to individual loci and to chromosomal regions within the mouse genome is the induction and/or the detection of insertional mutants.

An insertion-induced mutation within the genome can be defined as the disruption of a wild-type gene *X* (and hence of its function) by the integration of non-*X* DNA into or around the physical limits of gene *X*. One hallmark of insertion-induced mutations is that the mutant phenotype, since it is caused by the insertion, always cosegregates with the insertion in genetic tests; hence, the insertion serves as a molecular marker for the mutation.

The analysis of insertion-induced mutations has revolutionized the study of molecular genetics in many organisms by providing a means, with recombinant DNA techniques, to clone loci of interest in any genome by a method known as molecular tagging. Any gene identified by a mutation induced by an insertion element can be cloned from the mutant's genome if one has a clone of the DNA entity presumed to have induced the mu-

tation. This technique was first utilized by Bingham et al. (20) in cloning the white (*w*) locus in *Drosophila*. In mice, molecular access has been gained to several loci by identifying an insertion-induced mutation at those particular loci. Work with retrovirus-induced mutations has been particularly illuminating. Retroviruses must, as an obligate part of their life cycle, reverse transcribe their RNA genome into DNA and integrate into the host genome. Jenkins et al. (21) demonstrated that the dilute (*d*) mutation of the mouse, which causes a morphological change in the pigment-producing melanocyte, was associated with the integration of a member of the ecotropic class of endogenous murine leukemia viruses. Further work (22) showed that wild-type germ-line revertants of the *d* mutation lost the majority of the retroviral sequence in every case examined; hence, the integration of the virus into or around the *d* region of chromosome 9 induced the *d* mutation. DNA from the *d* region flanking the site of viral integration was isolated from genomic libraries prepared from DNA of *d/d* mice by using a specific subclone of the retroviral genome as a probe (22). Because the product of the *d* gene is not known, molecular access to the *d* locus and all of its genetic complexity (see next section) would have been very difficult to achieve if this spontaneous insertion mutation within the mouse genome had not been detected.

Analysis of proviral integration sites in mouse tumors has also been instrumental in identifying loci (oncogenes) within the genome that are associated with carcinogenesis. For example, Nusse and Varmus (23) found that a number of mammary tumors in C3H/He mice carried integrations of mouse mammary tumor virus (MMTV) at a common locus (*int-1*) within the genome. These proviral insertions may alter the normal expression of a potentially oncogenic locus (or loci) near the integration site (24). [Other oncogene loci have been identified in this manner; the reader is referred to the excellent reviews by Varmus (25) and Nusse (26) for a comprehensive treatment of this subject.]

Experimentally induced insertional mutations also hold great promise for the generating, tagging, and cloning of mutations that specify aberrant developmental phenotypes. One system that has already proved ideal for the generation of insertion-induced mutations is the production of transgenic mice. Transgenic mice are created by microinjection of small volumes of cloned DNA sequences into the male pronucleus of fertilized eggs, followed by reimplantation of the injected embryos into pseudopregnant females (27). At a certain frequency, the injected DNA integrates into the embryo's genomic DNA and can be inherited as a discrete unit(s) by all cells (including the germ line) of the developing individual. Mice carrying the injected DNA within their genome can easily be detected by Southern blot hybridization of their genomic DNA, using the cloned DNA that was used for injection as a probe. This technique has thus allowed stable, heritable gene transfer of cloned DNA from the test tube back into the whole animal and has revolutionized the study of develop-

mental and tissue expression of specific genes in the mouse (28).

An important observation made during the analysis of mice carrying specific injected genes at apparently random integration sites was that new mutant phenotypes sometimes appeared in certain lines carrying insertions at particular sites within the genome. Thus, integration of the injected DNA, a necessary event in the production of transgenic mice, can induce mutations in much the same way as integration of mobile elements or viral sequences can disrupt host gene function. Wagner et al. (29) have reported lines of transgenic mice that could not be made homozygous for the insertion they carried, even though the insertion could be inherited from both the female and male parent. Hence, these insertions act like recessive-lethal mutations, and most likely interrupt genes whose functions are absolutely required for normal embryogenesis. Palmiter et al. (30) have reported an insertion in a transgenic line that cannot be inherited through the male germ line, and these authors hypothesize that this particular insertion has interrupted the functioning of a critical gene normally expressed postmeiotically during male gametogenesis. Still another example is the insertion mutation reported by Woychik et al. (31) that is allelic to the limb deformity (*ld*) mutation on mouse chromosome 2, which identifies a gene required for the normal patterning of limb formation. Importantly, in all of these cases, the induced mutation is tagged by a molecular marker (the injected DNA itself), which enables direct molecular analysis of both mutant and wild-type forms of the region with recombinant DNA techniques. Table 2 summarizes some of the instances of insertional mutagenesis that are representative of the power of this technique for inducing and providing molecular access to mutant loci with defined developmental phenotypes.

Molecular Analysis of Mutations at the Dilute-Short Ear (*d-se*) Region of Chromosome 9: A Prototype

The molecular genetic analysis of the dilute-short ear (*d-se*) region of the mouse (19,34) serves well to illustrate some of the aforementioned principles and strategies for initiating a molecular characterization of the nature and effects of heritable mutations. Figure 1 presents a schematic summary of what is currently known about the genetic, physical, functional, and deletion maps of this small region of chromosome 9 (17,19). The original *d* mutation, a recessive variant of the mouse fancy (35), causes as its single phenotypic effect a dilution of coat color, which results from a change in melanocyte morphology and pigment distribution. However, incorporation of the *d* and closely linked *se* loci into a multiply marked specific-locus tester stock allowed the identification of hundreds of radiation- and chemical-induced mutations at these two loci (17). Wild-type animals, treated with a variety of radiations or chemicals, are crossed to this tester stock; heritable

Table 2. Examples of insertional mutagenesis.

Mutagen	Locus	Phenotype	Reference
Retrovirus (<i>Emv-3</i>)	<i>d</i> (dilute)	Altered melanocyte morphology	(21,22)
Retrovirus (<i>Mov-13</i>)	II (I) collagen	Recessive lethality	(32)
Microinjected cloned DNA	<i>ld</i> (limb deformity)	Skeletal abnormalities	(31)
Microinjected cloned DNA	?	Recessive lethality	(28,29)
Microinjected cloned DNA	?	Germ-line transmission only through female	(30)
Retrovirus (MMTV)	<i>int-1</i> (integration-1)	Common integration sites in mammary tumors	(23)
	<i>int-2</i> (integration-2)		(33)
Retrovirus (many)	<i>c-onc</i> (cellular oncogenes)	Associated with oncogenesis	(25,26)

recessive mutations, induced by the agent in the germ line of the wild-type parent, are subsequently recovered at the tester loci (two of which are *d* and *se*) in the next generation. The radiation-induced *d-se* mutations could be grouped by phenotype into several categories: (a) '*d*,' which were apparent repeat mutations to *d* that caused dilution of coat color as the single phenotypic effect; (b) *d^x* (dark dilute), which produced animals intermediate in color between dilute and wild-type; (c) '*se*,' which were apparent repeat mutations to *se*; (d) *se^x*, (short-ear intermediate), which produced animals with ear length intermediate between that specified by *se* and by wild-type; (e) *d^{pl}* (dilute-prenatal lethal), which produced dilute animals in the construction *d^{pl}/d*, but were prenatally lethal when homozygous; (f) *d^{op}* (dilute-opisthotonic), which produced dilute animals in the construction *d^{op}/d*, but were postnatally lethal when homozygous (these *d^{op}/d^{op}* dilute animals are characterized by opisthotonic convulsions and die before or at weaning); (g) *se^l* (short-ear lethal), which produced short-eared animals in the construction *se^l/se* but were prenatally lethal when homozygous; and (h) *Df(dse)* (deficiency-dilute-short-ear), which produced animals mutant at both *d* and *se* in constructions *Df(dse)/d se*, but were prenatally lethal when homozygous (17).

Pairwise complementation crosses between many independent representatives of each of these classes of radiation-induced *d-se* mutations have demonstrated that many can be interpreted as being multilocus deletions that involve the *d* and/or *se* loci themselves, as well as segments of chromosome between and/or immediately surrounding *d* and *se* (17). By ascertaining which mutations can complement for the various functions associated with the region (melanocyte morphology, opisthotonus, prenatal lethality, neonatal lethality, cartilage and ear morphology), the radiation mutations could be grouped into at least 16 complementation groups spanning at least five loci (functional complementation units) for prenatal lethality (*pl-1-5*); two loci for neonatal lethality (*nl-1* and 2); one for dilute and

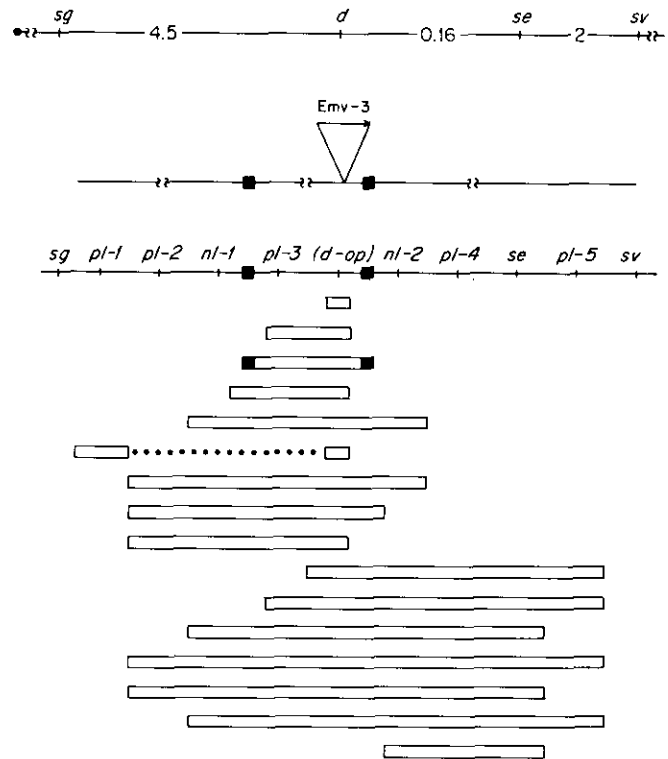


FIGURE 1. Analysis of the *d-se* region in mouse chromosome 9. Based on data from (9,17,19). Shown (from top to bottom) are the genetic (recombination), physical, and functional maps, as they stand to date, for the *d-se* region of chromosome 9. None of the map intervals are drawn to scale with respect to physical distance. Intervals on the genetic map are given in centimorgans (cM, 1% recombination); the centromeric end (toward the left) is indicated by (●). Genetic loci include *sg*, staggerer; *d*, dilute; *se* short ear; and *sv*, Snell's waltzer. On the physical map, the location (and direction of transcription) of the *Emv-3* endogenous retrovirus associated with the dilute (*d*) mutation is indicated. The functional map is drawn linearly, and no correlation with physical distance is implied. Additional functional units include *pl-1* through *pl-5*, prenatal lethals; *nl-1* and *nl-2*, neonatal lethals; and *op*, opisthotonus. Since *d* and *op* have not, to date, been separable by complementation or recombination, they are shown at the same site. Depicted below the functional map is a subset of the panel of deletion mutations analyzed both by complementation and by molecular probes (19). (□) represents the extent of the presumed deletion for individual mutants within complementation groups. (■) represents the cloned ends of a *d^{pl}* deletion mutation (19); the presumed map positions of these clones on the physical and functional maps are indicated, as is the particular deletion mutation from which these ends were cloned. Although these maps are not drawn to scale with respect to physical or genetic distance, the positions of the shaded boxes on the physical and functional maps accurately reflect the extent of deletions with respect to the cloned ends of the *d^{pl}* mutation (19). Intervals of the functional map skipped by a mutation that defines *pl-1* (17) are indicated by a (.....).

opisthotonus (*d* and *op*, not separable by complementation); one for cartilage frameworks (*se*); and one for another neurological disorder (*sv*, Snell's Waltzer), located some 2 cM distal to *se* (17,19). These functional units could be aligned, with few exceptions, in a linear complementation map (see Fig. 1), with the individual complementation groups representing varying extents

of deleted DNA in the radiation-induced *d-se* mutations (17,19). It should be noted that the deleted segments, which define functional units, cover stretches of DNA of undefined length; hence, the majority, if not all, of these segments almost certainly contains more than one gene.

Molecular analysis of the genomic organization of the *d-se* region, its radiation-induced mutations, and its associated developmental functions was made possible by the discovery that the original *d* mutation was caused by the integration of murine leukemia retroviral DNA (*Emv-3*) into or near the *d* locus (21). This viral sequence was used to clone a chromosome-9 unique-sequence DNA flanking the viral integration site in *d/d* animals (22), and this unique-sequence clone was then used to probe genomic DNA from a panel of *d-se* radiation mutants (19). The salient findings of this analysis were that many *d* radiation mutations inferred by complementation analysis to be deletions were, in fact, deleted for DNA immediately surrounding the proviral (*Emv-3*) integration site; a deletion breakpoint-fusion fragment was detected in, and cloned from, one *d^{pl}* radiation mutation; a unique-sequence clone, derived from the other end of this deletion breakpoint-fusion fragment was mapped between *nl-1* and *pl-3* on the complementation map; the rudimentary physical map of the region was correlated with the present functional (complementation) map; and the molecular probes were used to discriminate between individual mutations within genetically defined complementation groups, thus allowing direct correlations, within regions, of specific DNA fragments with specific phenotypes (19) (Fig. 1).

It is noteworthy that the *d-se* deletion mutations, along with the two molecular access points to *d-se*-region DNA (19,22), provide important tools for continuing the analysis of this region of the mouse genome. Nonetheless, some important information has already come from the molecular and genetic analysis of the *d-se* region and from similar genetic analyses performed in other chromosomal regions associated with radiation-induced deletion mutations. For example, it is possible to map DNA clones with respect to deletion breakpoints in order to initiate a finer physical map of a small chromosomal segment. One can anticipate being able to perform similar types of mapping analyses with region-specific probes and panels of deletion mutations, which can result in placing cloned DNA probes onto a functional map of a region. Likewise, cloning of deletion breakpoint-fusion fragments allows large chromosomal jumps within regions, thus facilitating the development of a physical map. It is also interesting to note that genetic analysis of mutations such as these provide striking evidence for many loci additional to those already found on the linkage map of the mouse genome. Consider that of the loci found on the *d-se* functional map at present (Fig. 1), only *sg*, *d*, *se*, and *sv* have previously been recognized as mutant loci in laboratory mice. The other functions now associated with this chromosomal region were recognized only after complementation analysis of the *d-se* deletion mutations; and even

these functional units probably underestimate greatly the number of distinct genes within this region. Thus, in the context of utilizing mutations at genetic loci as biomarkers for developmental processes, the number of presently recognized loci will grossly underestimate the total number of genes that must function within the organism. This concept should be considered when one evaluates the use of (and molecular characterization of) heritable mutations as biomarkers for reproductive and developmental toxicology—the target is large and is composed of many diverse parts, and many effects relating to exposure may never be detected.

Concluding Remarks

Clearly, this is an exciting time in mammalian genetics. Strategies in which the analytical tools of molecular biology are combined with the techniques and resources provided by genetic analysis have great potential both for defining the molecular structure and expression of wild-type and mutant genes and for relating structure and expression to function and phenotype. Studies of the developmental regulation of specific genes in the context of the interaction of segments of DNA with their nuclear, cellular, and tissue environments are proceeding with scores of illuminating results. Transgenic mouse technology, which has been crucial to these studies, is also contributing to basic genetic analysis by providing a useful insertional mutagenesis system as well as a useful gene-transfer system. The latter may provide a means for the ultimate identification of cloned DNA segments as specific genes by correction-of-mutant-phenotype experiments in the whole organism. Some of the technical and conceptual roadblocks now associated with many of the avenues of inquiry outlined in Table 1 may therefore be eliminated by further analysis of mutations within experimentally malleable mouse systems, thus complementing, and contributing to, the study of human genetics.

Exploitation of radiation-induced deletion mutations of the mouse for initiating and refining the molecular and functional characterization of entire chromosomal regions has the potential for broadening our perspective on the genetic organization of specific segments of the mammalian genome. This type of analysis should result in an understanding of the structure and expression of currently recognized genes and gene products (both wild-type and altered), and their roles in development. It should also result in the genetic and molecular definition of additional genes, which otherwise might be difficult or impossible to detect, as well as in an understanding of the types (and effects) of genomic lesions induced by different mutagenic treatments.

On a different front, the intriguing and very basic question of the nature and effects of dominant mutations also deserves some consideration, both in the context of biomarkers for exposure and/or effects and in the context of molecular analysis of heritable mutations. Since dominant mutations express their phenotypes in a heterozygous, one-dose state, they are often the most

important type of aberration considered for genetic risk estimation. In the mouse, a wide spectrum of dominant mutations, both in terms of gross nature and in terms of effect, has been observed. Admittedly, the natures of most of these mutations remain unclear. However, at least two dominant mutations have been associated with deletions [T^{hp} , Brachyury-hairpin (36); W^{19H} , Dominant spotting-19H (37)] and others have been associated with translocation breakpoints [five *Sl* (Steel) mutations (38, and L. B. Russell, unpublished observations) and the "Diver" neurological mutation (39)], much the same as a dominant phenotype of oncogenesis is often associated with translocations in somatic cells (25).

The phenotypic effects of dominant mutations rarely are simple. Often, as is the case for mutations like *W* (dominant spotting), *A^y* (lethal yellow), *Sl*, and a large number of heritable skeletal mutations, several distinct body systems (and thus, presumably, distinct physiological and developmental processes) are affected by a single pleiotropic mutation. Consequently, molecular analysis of these types of mutations may add not only to an understanding of the structural nature of the mutation and the reason why its effects are dominant, but also to an understanding of the interrelationships between developmental pathways throughout the life cycle of mammals.

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